Message

From: Detlef Knappe [knappe@ncsu.edu]
Sent: 7/19/2019 2:22:41 PM
To: Strynar, Mark [Strynar.Mark@epa.gov]
CC: David Muddiman [dcmuddim@ncsu.edu]; Jane Hoppin [jahoppin@ncsu.edu]; Jeffrey Enders [jrenders@ncsu.edu]; Nadine Kotlarz [nkotlar@ncsu.edu]
Subject: Re: Hoppin PFC method

Even for the low temp method, Zack starts with M-H-CO2 for the perfluoroethercarboxylic acids. The ion is much more abundant relative to M-H. If Jeff has questions, Zack and Noelle are happy to help.

For targeted work, here are our retention times and transitions (for most compounds, we have two transitions, but there are some, for which we can get only one):

Name (Acronym)	Precursor Ion	Confirmation Ion	RT (min)	Fragmentor (V)	Collision Energy (V)	Capillary Voltage (V)	IS
High Temperature Ion Source Compounds							
PFBA (1)	213.0	169.0	5.014	84.0	0.0	2000	MPFBA
NVHOS (1)	296.9	79.9	6.648	140.0	48.0	2000	M3PFBS
NVHOS (2)	269.9	135.1	6.648	140.0	24.0	2000	M3PFBS
Nafion Byproduct 4 (1)	440.9	197.0	5.698	140.0	32.0	2000	MPFBA
Nafion Byproduct 4 (2)	440.9	241.0	5.698	140.0	20.0	2000	MPFBA
PFPeA (1)	263.0	219.0	7.545	80.0	0.0	2000	MPFPeA
PFBS (1)	298.9	79.9	7.814	140.0	40.0	2000	M3PFBS
PFBS (2)	298.9	98.8	7.814	140.0	28.0	2000	M3PFBS
4:2FTS (1)	327.0	307.0	8.510	110.0	16.0	2000	M2-4:2FTS
4:2FTS (2)	327.0	80.8	8.510	110.0	32.0	2000	M2-4:2FTS
PFHxA (1)	313.0	268.9	8.564	94.0	0.0	2000	MPFHxA
PFHxA (2)	313.0	119.0	8.564	94.0	16.0	2000	MPFHxA
PFPeS (1)	348.9	79.9	8.647	125.0	52.0	2000	MPFHxS
PFPeS (2)	348.9	99.0	8.647	125.0	36.0	2000	MPFHxS
PFHpA (1)	363.0	319.0	9.205	100.0	0.0	2000	MPFHpA
PFHpA (2)	363.0	169.1	9.205	100.0	8.0	2000	MPFHpA
PFHxS (1)	398.9	79.8	9.228	166.0	68.0	2000	MPFHxS
PFHxS (2)	398.9	98.9	9.228	166.0	36.0	2000	MPFHxS
Nafion Byproduct 2 (1)	462.9	263.0	9.240	140.0	24.0	2000	MPFHxS
Nafion Byproduct 2 (2)	462.9	213.0	9.240	140.0	32.0	2000	MPFHxS
Nafion Byproduct 1 (1)	442.9	263.0	9.540	140.0	12.0	2000	MPFHxS
Nafion Byproduct 1 (2)	442.9	147.1	9.540	140.0	24.0	2000	MPFHxS
6:2FTS (1)	427.0	406.9	9.670	160.0	20.0	2000	M2-6:2 FTS
6:2 FTS (1)	427.0	80.9	9.670	160.0	40.0	2000	M2-6:2 FTS
PFHpS (1)	448.9	79.8	9.677	160.0	60.0	2000	MPFHxS
PFHpS (2)	448.9	98.8	9.677	160.0	44.0	2000	MPFHxS
PFOA (1)	413.0	369.0	9.679	103.0	0.0	2000	MPFOA
PFOA (2)	413.0	168.9	9.679	103.0	12.0	2000	MPFOA
PFOS (1)	498.9	79.7	10.051	170.0	76.0	2000	MPFOS
PFOS (2)	498.9	98.8	10.051	170.0	44.0	2000	MPFOS
PFNA (1)	463.0	418.9	10.067	100.0	0.0	2000	MPFNA
PFNA (2)	463.0	219.0	10.067	100.0	8.0	2000	MPFNA
8:2FTS (1)	527	506.9	10.342	120.0	24.0	2000	M2-8:2FTS
8:2FTS (2)	527	80.8	10.342	120.0	56.0	2000	M2-8:2FTS
PFDA (1)	513.0	469.0	10.387	120.0	0.0	2000	MPFDA
PFDA (2)	513.0	269.0	10.387	120.0	8.0	2000	MPFDA
PFNS (1)	549.0	80.0	10.405	100.0	60.0	2000	MPFOS
PFNS (2)	549.0	99.0	10.405	100.0	45.0	2000	MPFOS
nMeFOSAA (1)	570.0	418.9	10.480	115.0	15.0	2000	D3-nMeFOSAA
nMeFOSAA (2)	570.0	482.9	10.480	115.0	12.0	2000	D3-nMeFOSAA

FOSA (1)	497.9	77.8	10.609	96.0	40.0	2000	MFOSA
FOSA (2)	497.9	477.8	10.609	96.0	20.0	2000	MFOSA
nEtFOSAA (1)	584.0	418.9	10.618	115.0	15.0	2000	D5-nEtFOSAA
nEtFOSAA (2)	584.0	525.9	10.618	115.0	12.0	2000	D5-nEtFOSAA
PFUdA (1)	563.0	519.0	10.618	72.0	4.0	2000	MP7FUdA
PFUdA (2)	563.0	268.9	10.618	72.0	12.0	2000	M7PFUdA
PFDS (1)	598.9	80.0	10.666	100.0	60.0	2000	MPFOS
PFDS (2)	598.9	99.0	10.666	100.0	45.0	2000	MPFOS
PFDoA (1)	613.0	568.9	10.851	78.0	4.0	2000	MPFDoA
PFDoA (2)	613.0	268.9	10.815	78.0	12.0	2000	MPFDoA
PFTrDA (1)	663.0	618.9	11.060	78.0	4.0	2000	M2PFTeDA
PFTrDA (2)	663.0	169.0	11.060	78.0	24.0	2000	M2PFTeDA
PFTeDA (1)	712.9	669.0	11.247	100.0	7.0	2000	M2PFTeDA
PFTeDA (2)	712.9	169.0	11.247	100.0	23.0	2000	M2PFTeDA
Low Temperature Ion Source Compounds							
PFMOAA (1)	179.0	85.0	2.651	79.0	4.0	1500	MPFBA
PFMOAA (2)	179.0	135.0	2.651	79.0	0.0	1500	MPFBA
PMPA (1)	229.0	184.9	5.721	89.0	0.0	1500	MPFBA
PMPA (2)	229.0	85.1	5.721	89.0	16.0	1500	MPFBA
PFO2HxA (1)	245.0	85.0	7.189	95.0	0.0	2000	MHFPO-DA
PFO2HxA (2)	201.0	85.0	7.189	95.0	0.0	2000	MHFPO-DA
PEPA (1)	235.0	135.0	7.877	100.0	16.0	2000	MHFPO-DA
PEPA (2)	279.0	235.0	7.877	100.0	0.0	2000	MHFPO-DA
PFO3OA (1)	311.0	85.0	8.692	105.0	0.0	2500	MHFPO-DA
PFO3OA (2)	311.0	151.0	8.692	105.0	0.0	2500	MHFPO-DA
HFPO-DA (1)	285.0	169.0	8.795	108.0	0.0	2500	MHFPO-DA
HFPO-DA (2)	329.0	169.1	8.795	108.0	4.0	2500	MHFPO-DA
HydroEve (1)	427.0	283.0	9.225	100.0	4.0	2500	MHFPO-DA
HydroEve (2)	427.0	262.9	9.225	100.0	12.0	2500	MHFPO-DA
ADONA (1)	377.0	250.9	9.293	70.0	0.0	2500	MHFPO-DA
ADONA (2)	377.0	84.9	9.293	70.0	0.0	2500	MHFPO-DA
PFO4DA (1)	376.9	85.0	9.459	110.0	0.0	2500	MHFPO-DA
PFO4DA (2)	376.9	150.9	9.459	110.0	0.0	2500	MHFPO-DA
PFO5DoA (1)	442.9	84.8	9.990	110.0	0.0	4000	MPFNA
PFO5DoA (2)	442.9	150.8	9.990	110.0	0.0	4000	MPFNA
F53B (1)	530.9	350.9	10.237	135.0	20.0	4000	MPFNA
F53B (2)	530.9	82.8	10.237	135.0	24.0	4000	MPFNA
Internal Standards							
MPFBA (1)	217.0	172.0	5.014	85.0	0.0	2000	
MPFPeA (1)	268.0	223.0	7.545	55.0	0.0	2000	
M3PFBS (1)	302.0	79.9	7.814	120.0	40.0	2000	
M3PFBS (2)	302.0	98.9	7.814	120.0	32.0	2000	
M2-4:2FTS (1)	329.0	308.9	8.510	105.0	16.0	1500	
M2-4:2FTS (2)	329.0	80.9	8.510	105.0	32.0	1500	
MPFHxA (1)	315.0	270.0	8.564	95.0	0.0	1500	
MPFHxA (2)	315.0	118.9	8.564	95.0	16.0	1500	
MHFPO-DA (1)	287.0	169.0	8.795	108.0	0.0	2500	
MFHPO-DA (2)	332.0	169.0	8.795	108.0	4.0	2500	
MPFHpA (1)	367.0	322.0	9.205	60.0	0.0	1500	
MPFHpA (2)	367.0	169.8	9.205	60.0	12.0	1500	
MPFHxS (1)	402.9	83.8	9.228	170.0	56.0	1500	
MPFHxS (2)	402.9	102.8	9.228	170.0	40.0	1500	
M2-6:2FTS (1)	429.0	409.0	9.670	165.0	20.0	1500	
M2-6:2FTS (2)	429.0	80.8	9.670	165.0	40.0	1500	
MPFOA (1)	417.0	372.0	9.679	105.0	0.0	1500	
MPFOA (2)	417.0	169.0	9.679	105.0	12.0	1500	
MPFOS (1)	502.9	79.8	10.051	190.0	60.0	1500	
MPFOS (2)	502.9	98.9	10.051	190.0	52.0	1500	
MPFNA (1)	468.0	423.0	10.067	105.0	0.0	1500	
MPFNA (2)	468.0	219.0	10.067	105.0	8.0	1500	
M2-8:2FTS (1)	529.0	508.9	10.342	110.0	24.0	1500	
M2-8:2FTS (2)	529.0	80.9	10.342	110.0	60.0	1500	
MFPDA (1)	515.1	470.0	10.387	110.0	0.0	1500	

MPFDA (2)	515.1	220.1	10.387	110.0	12.0	1500	
D3-nMeFOSAA (1)	573.0	418.9	10.480	90.0	12.0	1500	
D3-nMeFOSAA (2)	573.0	482.9	10.480	90.0	8.0	1500	
MFOSA (1)	506.0	77.8	10.609	108.0	44.0	1500	
MFOSA (2)	506.0	485.9	10.609	108.0	20.0	1500	
D5-nEtFOSAA (1)	589.0	419.0	10.618	90.0	12.0	1500	
D5-nEtFOSAA (2)	589.0	531.0	10.618	90.0	16.0	1500	
M7PFUdA (1)	570.0	524.9	10.618	66.0	0.0	1500	
M7PFUdA (2)	570.0	270.0	10.618	66.0	12.0	1500	
MPFDoA (1)	615.0	570.0	10.851	72.0	4.0	1500	
MPFDoA (2)	615.0	320.0	10.851	72.0	12.0	1500	
M2PFTeDA (1)	715.0	670.0	11.247	78.0	4.0	1500	
M2PFTeDA (2)	715.0	169.1	11.247	78.0	24.0	1500	

Best,
Detlef

On Fri, Jul 19, 2019 at 7:25 AM Strynar, Mark <Strynar.Mark@epa.gov> wrote:

All,

We have seen this as well on our MS systems. The fluoroethers decarboxylate readily at lower temp and voltage compared to other PFCAs and PFSAs. One work around it to monitor for the decarboxylated ion as the primary. Then you may only get one ion as the primary MRM is CO2 loss. I see this is what many contact labs are doing when I see their methods. We also find the perfluoro-ethers readily form gas phase H+ and Na+ dimers, with the M-H- ion being small to non-existant.

We also know that HFPO-DA and at least 2 others we know of HFPO-TA, HFPO-TetA are not stable in DMSO. They turn into H substituted perfluoro-ethers in the carboxylate position. I expect PMPA, and PEPA will do the same.

Mark

From: Detlef Knappe <knappe@ncsu.edu>
Sent: Thursday, July 18, 2019 9:57 PM
To: David Muddiman <dcmuddim@ncsu.edu>
Cc: Jane Hoppin <jahoppin@ncsu.edu>; Strynar, Mark <Strynar.Mark@epa.gov>; Jeffrey Enders <jrenders@ncsu.edu>; Nadine Kotlarz <nkotlar@ncsu.edu>
Subject: Re: Hoppin PFC method

Hi Dave,

The main consideration is sensitivity. The sulfonic acids ionize better at higher temp, giving us lower reporting limits. But at the higher temp, you obliterate the fluoroether carboxylic acids. So we have to run those at a lower temp. Lee Ferguson is seeing the same and is running both the low and high temp method to get the reporting limits we need. On an instrument with high sensitivity, it may be possible to just run at low temp, and I have asked Becca to check reporting limits she can get for all compounds using the low temp method. Please let us not reinvent things from scratch - we have been doing this for quite some time now. We need to make

progress on samples. The days between now and July 29 are absolutely critical for getting results. If we spend more time on method development, we will be going to conferences in August and have nothing to report.

Another thing we just learned is that the branched ethers (PMPA, PEPA, GenX) are not stable in acetonitrile. We need to make all standards in methanol. And for carboxylic acids we need to use basic methanol to prevent the formation of methyl esters.

Best,

Detlef

On Thu, Jul 18, 2019 at 6:41 PM David Muddiman <dcmuddim@ncsu.edu> wrote:

Hi Mark

Perhaps you, James and my folks should have a talk about things. We are finding the analytical side of things to be strange. Does not make sense that compounds under gradient elution would have vastly different desolation temperatures given the dominate factor is solvent comp. how can this be? There is something strange here. Need to figure out ASAP. In other words why don't the compounds at higher temp work at lower temps.

And big question why with "The Devil We Know" are we still studying this after 30 years. It is known there are health efforts from PFOS and GenX. Hmmmmm

Dave

Sent from my iPhone, Please forgive brevity and typos :-)

On Jul 18, 2019, at 6:24 PM, Jane Hoppin <jahoppin@ncsu.edu> wrote:

Hi Dave

I'm including Mark Strynar since the work was done in his lab, so I'm sure he'll have some thoughts about the solvent issue

Cheers

Jane

On Thu, Jul 18, 2019 at 5:58 PM David Muddiman <dcmuddim@ncsu.edu> wrote:

Hi Nadine,

First, the low temp and high temp methods are curious to me. This should never be the case on a MS system. Something strange here going on. Solvent is solvent. So, while it might work, it does not make sense to me. I need to sort this out.

Second, just adding this and that and this and that, means an entirely new method. We need to know what you want to measure. We can get compound with suspect concentrations and some with semi-reliable concentrations and "run the samples". We need to know what matter and do significant due diligence to make sure we can provide accurate numbers versus just numbers. So, the less we have to develop and QC/QA the sooner we can make this happen. Lots of samples and lots of analytes.

Please advise, not just to Nadine but to Detlef and Jane too.

Nadine, I saw you in Whole Foods yesterday but I knew I knew you but could not piece it together until your email. Safe travels,

Dave

On Thu, Jul 18, 2019 at 4:57 PM Jane Hoppin <jahoppin@ncsu.edu> wrote:

Thanks Nadine!

On Thu, Jul 18, 2019 at 3:54 PM Nadine Kotlarz <nkotlar@ncsu.edu> wrote:

Hi Jeff,

We should start with, at a minimum, the 28 PFAS that are covered collectively on our Ultivo QQQ low temperature and high temperature methods. Here's the list

PFAS with standards from Chemours:

1. PFMOAA
2. PEPA
3. PMPA
4. PFO2HxA
5. PFO3OA
6. GenX
7. NVHOS
8. PFO4DA
9. Hydro-EVE
10. PFO5DoA
11. Nafion byproduct 1
12. Nafion byproduct 2
13. Nafion byproduct 4

PFAS with standards that can be purchased from Wellington:

1. PFBA
2. PFBS
3. PFPeA

4. PFPeS
5. PFHxA
6. PFHxS
7. PFHpA
8. PFHpS
9. PFOA
10. PFOS
11. PFNA
12. PFDA
13. 4:2FTS
14. 6:2FTS
15. 8:2FTS

We have some more standards from Chemours that didn't make it into the Ultivo method but may be good to incorporate into your method on the Altis. Those are the ones highlighted in blue in the attached doc.

We've also been using 20 internal standards for the analysis. We purchase one mix with 19 internal standards and MGenX separately. Invoice from a past purchase attached.

I'm out of town today and tomorrow but back in the office on Monday.

Nadine

On Thu, Jul 18, 2019 at 2:42 PM Jeffrey Enders <jrenders@ncsu.edu> wrote:

> Hi Nadine,
>
> Can I get confirmation from you on the list provided below? I am trying to get these nailed down so that I can make sure we have all of the standards and then order the ones that we don't have and get started on method development. I am basing this list on a table from the document attached. This document was given to Allison and is posted to this project on MENDIX. Thanks.

1	GenX
2	Nafionbp1
3	Nafionbp2
4	Nafionbp4
5	PFO2HxA
6	PFO3OA
7	PFO4DA

8	PFO5DoDA
9	PMPA
10	NVHOS
11	PEPA
12	PFBA
13	PFPeA
14	PFHxA
15	PFHpA
16	PFOA
17	PFNA
18	PFDA
19	PFBS
20	PFHxS
21	PFOS
22	6:2_FTS

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On Tue, Jul 16, 2019 at 3:25 PM Jane Hoppin <jahoppin@ncsu.edu> wrote:

We also are interested in Hydro-Eve and we also have a standard for that.

Seems like we looked for 24, so want Nadine to weigh in, in case I missed one.

Thanks.

On Tue, Jul 16, 2019 at 2:57 PM Jeffrey Enders <jrenders@ncsu.edu> wrote:

Hi Jane,

Thanks I found the document on MENDIX, as you suggested. Are the 22 compounds in that document the ones you are interested in analyzing for in these samples as well (see table below)? Sample prep will be the same, but the main difference between the orbitrap and the QQQ is that you have to decide what analytes you want to look for before running the samples. The QQQ is also inherently more suited to quantitation (most would argue).

Thanks for the heads up on the nomenclature - I thought it was PFAS but saw Wellington refer to their catalog section as PFC so incorrectly altered my language.

Thanks.

1	GenX
2	Nafionbp1
3	Nafionbp2
4	Nafionbp4
5	PFO2HxA
6	PFO3OA
7	PFO4DA
8	PFO5DoDA
9	PMPA
10	NVHOS
11	PEPA
12	PFBA
13	PFPeA
14	PFHxA
15	PFHpA

16	PFOA
17	PFNA
18	PFDA
19	PFBS
20	PFHxS
21	PFOS
22	6:2_FTS

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On Tue, Jul 16, 2019 at 2:41 PM Jane Hoppin <jahoppin@ncsu.edu> wrote:

Hey Jeff,

I'm excited to see you working on this. We already shared our blood protocol with Allison, so you should review that, so you won't be starting brand new. Someone should have shared those with you and you should work with those. I know there will be some differences between the QQQ and the orbitrap, but the sample preparation should be the same.

FYI, we call these PFAS and not PFCs (PFCs include the fluorochemicals that damage the ozone layer).

Please let me know if you need the document I previously sent Allison. I thought she was going to upload into Mendix

Thanks.

Jane

On Tue, Jul 16, 2019 at 2:35 PM Jeffrey Enders <jrenders@ncsu.edu> wrote:

> Hi Jane and Nadine,
>
> Dave and I have met and I will begin working on a PFC method for your blood samples. I don't have much information on which compounds you are primarily interested in and this will have a significant impact on the time, effort requirement, and feasibility of this study. I have been collecting information from folks about what standards we have, what methods we have already developed, and what protocols have already been written up. I will try to summarize what is available and try to get from your which compounds you are hoping to quantify.
>
> I will primarily be building off of protocols that Detlef's lab already runs and an instrument method that was shared by Duke and has been partially set up on our instrument. The protocol that Zack Hopkins has shared with me lists the following compounds as being detectable:

Chemical Name	Acronym	Isomer	Internal Standard
Perfluoro-n-butanoic acid	PFBA	linear	MPFBA
Perfluoro-n-pentanoic acid	PFPeA	linear	M5PFPeA
Perfluoro-n-hexanoic acid	PFHxA	linear	M5PFHxA
Perfluoro-n-heptanoic acid	PFHpA	linear	M4PFHpA
Perfluoro-n-octanoic acid	PFOA	linear	M8PFOA
Perfluoro-n-nonanoic acid	PFNA	linear	M9PFNA
Perfluoro-n-decanoic acid	PFDA	linear	M6PFDA
Perfluoro-n-undecanoic acid	PFUnDA	linear	M7PFUnDA
Perfluoro-n-dodecanoic acid	PFDoDA	linear	MPFDoDA
Perfluoro-n-tridecanoic acid	PFTrDA	linear	N/A – use M2PFTeDA
Perfluoro-n-tetradecanoic acid	PFTeDA	linear	M2PFTeDA
Perfluorobutane sulfonate	PFBS	linear	M3PFBS
Perfluoropentane sulfonate	PFPeS	linear	N/A – use M3PFHxS
Perfluorohexane sulfonate	PFHxS	linear / branched	M3PFHxS
Perfluoroheptane sulfonate	PFHpS	linear	N/A – use M3PFHxS
Perfluorooctane sulfonate	PFOS	linear / branched	M8PFOS
Perfluorononane sulfonate	PFNS	linear	MPFNS
Perfluorodecane sulfonate	PFDS	linear	MPFDS
Perfluorooctane sulfonamide	PFOSA	linear	M8FOSA-I
N-methylfluorooctance sulfonamido acetic acid	N-MeFOSAA	linear	d3-N-MeFOSAA
N-ethylfluorooctance sulfonamido acetic acid	N-EtFOSAA	linear	d5-N-EtFOSAA
4:2 fluorotelomer sulfonate	4:2 FTS	linear	M2-4:2 FTS
6:2 fluorotelomer sulfonate	6:2 FTS	linear	M2-6:2 FTS
8:2 fluorotelomer sulfonate	8:2 FTS	linear	M2-8:2 FTS

<image.png>

If we stick to the first table alone, the method development step will progress much more quickly as these compounds are sold by Wellington as a mixture and so can easily be made into a calibration curve. The second table is made manually by adding all compounds one at a time and so this will increase complexity. All of the compounds in these two tables are in the method that we are working to set up on the instrument. Additionally, Wellington and Cambridge isotope labs sell additional PFC compounds. There are far too many to list here but the links can be found below:

- https://well-labs.com/wellingtoncatalogue1618.html (starting on page 140)
- https://shop.isotope.com/category.aspx?id=10032748

Adding compounds to the method beyond the tables listed in this email, while possible, will increase the complexity of the method and inherently increase the risk of internal interferences (i.e., one compound enhances or suppressed the signal of another compound in method). The method that the Knappe group use is about 20 min long and adding compounds may also necessitate making this method longer due to instrument scan speed issues.

Any information you have on compounds of interest or other thoughts or concerns would help guide the conversation. I'm looking forward to working with you on this. Thanks!

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